CLAIMS

1. A purified polynucleotide wherein said polynucleotide is chosen from the group consisting of:

a) a polynucleotide comprising the following nucleotide sequence of SEQ ID NO 1:

CTGCAGCAGGTGACGTCGTTGTTCAGCCAGGTGGGCGGCACCGGCGGCGG
CAACCCAGCCGACGAGGAAGCCGCGCAGATG
GGCCTGCTCGGCACCAGTCCGCTGTCGAACCATCCGCTGGCTGGTGGATC
AGGCCCCAGCGCGGGCGCGGGCCTGCTGCG
CGCGGAGTCGCTACCTGGCGCAGGTGGGTCGTTGACCCGCACGCCGCTGA
TGTCTCAGCTGATCGAAAAGCCGGTTGCCC
CCTCGGTGATGCCGGCGGCTGTTGCCGGATCGTCGGTGACGGGTGGCGCC
GCTCCGGTGGGTCCGGGAGCGATGGGCCAG
GGTTCGCAATCCGGCGGCTCCACCAGCCCGGGTCTGGTCGCGCCGGCACC
GCTCGCGCAGGAGCGTGAAGAAGACGACGA
GGACGACTGGGACGAAGAGGACGACTGGTGAGCTCCCGTAATGACAACA
GACTTCCCGGCCACCCGGGCCGGAAGACTTG
CCAACATTTTGGCGAGGAAGGTAAAGAGAGAAAGTAGTCCAGCATGGCAG
AGATGAAGACCGATGCCGCTACCCTCGGGC
AGGAGGCAGGTAATTTCGAGCGGATCTCCGGCGACCTGAAAACCCAGATC
GACCAGGTGGAGTCGACGGCAGGTTCGTTG
CAGGGCCAGTGGCGCGGCGCGGCGGGGACGGCCGCCCAGGCCGCGGTGG
TGCGCTTCCAAGAAGCAGCCAATAAGCAGAA
GCAGGAACTCGACGAGATCTCGACGAATATTCGTCAGGCCGGCGTCCAAT
ACTCGAGGGCCGACGAGGAGCAGCAGCAGG
CGCTGTCCTCGCAAATGGGCTTCTGACCCGCTAATACGAAAAGAAACGGA
GCAAAAACATGACAGAGCAGCAGTGGAATT
TCGCGGGTATCGAGGCCGCGGCAAGCGCAATCCAGGGAAATGTCACGTCC
ATTCATTCCCTCCTTGACGAGGGGAAGCAG

TCCCTGACCAAGCTCGCAGCGGCCTGGGGCGGTAGCGGTTCGGAGGCGTA
CCAGGGTGTCCAGCAAAAATGGGACGCCAC
GGCTACCGAGCTGAACAACGCGCTGCAGAACCTGGCGCGGACGATCAGCG
AAGCCGGTCAGGCAATGGCTTCGACCGAAG
GCAACGTCACTGGGATGTTCGCATAGGGCAACGCCGAGTTCGCGTAGAAT
AGCGAAACACGGGATCGGGCGAGTTCGACC
TTCCGTCGGTCTCGCCCTTTCTCGTGTTTATACGTTTGAGCGCACTCTGAG
AGGTTGTCATGGCGGCCGACTACGA

b) a polynucleotide comprising the following nucleotide sequence of SEQ ID NO 2, starting at its 5' end with the nucleotide in position 1 of SEQ ID NO 1 and ending at its 3' end with the nucleotide in position 524 of SEQ ID NO 1, or a biologically active polynucleotide derivative of SEQ ID NO 2:

CTGCAGCAGGTGACGTCGTTGTTCAGCCAGGTGGGCGGCACCGGCGGCGG
CAACCCAGCCGACGAGGAAGCCGCGCAGATG
GGCCTGCTCGGCACCAGTCCGCTGTCGAACCATCCGCTGGCTGGTGGATC
AGGCCCCAGCGCGGGCGCGGGCCTGCTGCG
CGCGGAGTCGCTACCTGGCGCAGGTGGGTCGTTGACCCGCACGCCGCTGA
TGTCTCAGCTGATCGAAAAGCCGGTTGCCC
CCTCGGTGATGCCGGCGGCTGTTGCCGGATCGTCGGTGACGGGTGGCGCC
GCTCCGGTGGGTCCGGGAGCGATGGGCCAG
GGTTCGCAATCCGGCGGCTCCACCAGCCCGGGTCTGGTCGCGCCGGCACC
GCTCGCGCAGGAGCGTGAAGAAGACGACGA
GGACGACTGGGACGAAGAGGACGACTGGTGAGCTCCCGTAATGACAACA
GACTTCCCGGCCACCCGGGCCGGAAGACTTG
CCAACATTTTGGCGAGGAAGGTAAAGAGAGAAAGTAGTCCAGC

c) a polynucleotide comprising the following nucleotide sequence of SEQ ID NO 3, starting at its 5' end with the nucleotide in position 1 of SEQ ID NO 1 and ending at its 3' end with the nucleotide in position 481 of SEQ ID NO 1, or a biologically active polynucleotide derivative of SEQ ID NO 3:

CTGCAGCAGGTGACGTCGTTGTTCAGCCAGGTGGGCGGCACCGGCGGCGG
CAACCCAGCCGACGAGGAAGCCGCGCAGATG

GGCCTGCTCGGCACCAGTCCGCTGTCGAACCATCCGCTGGCTGGTGGATC
AGGCCCCAGCGCGGGCGCGGGCCTGCTGCG
CGCGGAGTCGCTACCTGGCGCAGGTGGGTCGTTGACCCGCACGCCGCTGA
TGTCTCAGCTGATCGAAAAGCCGGTTGCCC
CCTCGGTGATGCCGGCGGCTGTTGCCGGATCGTCGGTGACGGGTGGCGCC
GCTCCGGTGGGTCCGGGAGCGATGGGCCAG
GGTTCGCAATCCGGCGGCTCCACCAGCCCGGGTCTGGTCGCGCCGGCACC
GCTCGCGCAGGAGCGTGAAGAAGACGACGA
GGACGACTGGGACGAAGAGGACGACTGGTGAGCTCCCGTAATGACAACA
GACTTCCCGGCCACCCGGGCCGGAAGACTTG

d) a polynucleotide comprising the following nucleotide sequence of SEQ ID NO 4, starting at its 5' end with the nucleotide in position 525 of SEQ ID NO 1 and ending at its 3' end with the nucleotide in position 826 of SEQ ID NO 1 coding for the LHP polypeptide:

ATGGCAGAGATGAAGACCGATGCCGCTACCCTCGGGC
AGGAGGCAGGTAATTTCGAGCGGATCTCCGGCGACCTGAAAACCCAGATC
GACCAGGTGGAGTCGACGGCAGGTTCGTTG
CAGGGCCAGTGGCGCGGCGCGGCGGGGACGGCCGCCCAGGCCGCGGTGG
TGCGCTTCCAAGAAGCAGCCAATAAGCAGAA
GCAGGAACTCGACGAGATCTCGACGAATATTCGTCAGGCCGGCGTCCAAT
ACTCGAGGGCCGACGAGGAGCAGCAGCAGG
CGCTGTCCTCGCAAATGGGCTTCTG

e) a polynucleotide comprising at least 12 consecutive nucleotides of a polynucleotide chosen among the group consisting of SEQ ID NO2, SEQ ID N 3 or SEQ ID NO 4;

f) A polynucleotide having a sequence fully complimentary to a polynucleotide chosen among the group consisting of SEQ ID NO 2, SEQ ID NO 3 or SEQ ID NO 4;

g) A polynucleotide hybridizing under stringent hybridization conditions with polynucleotide chosen among the group consisting of SEQ ID NO 2, SEQ ID NO 3 or SEQ ID NO 4.

2. A polynucleotide according to Claim 1 wherein said polynucleotide codes for an antigenic protein from Mycobacterium tuberculosis comprising the following amino acid sequence of SEQ ID NO 4:

MAEMKTDAATLGQEAGNFERISGDLKTQIDQVESTAGSLQGQWRGAAGTAA
QAAVVRFQEAANKQKQELDEISTNIRQAGVQYSRADEEQQQALSSQMGF

3. A polynucleotide according to Claim 1 which is labeled with a marker compound.

4. A purified polynucleotide comprising:

a) a polynucleotide of sequence SEQ ID NO 2 or a biologically active polynucleotide derivative of SEQ ID NO 2; and

b) a polynucleotide coding for a polypeptide.

5. A purified polynucleotide comprising:

a) a polynucleotide of sequence SEQ ID NO 3 or a biologically active polynucleotide derivative of SEQ ID NO 2; and

b) a polynucleotide coding for a polypeptide.

6. A recombinant vector containing a polynucleotide according to ~~any one of Claims 1 to 5~~ Claim 1.

7. The recombinant vector according to Claim 6, which is plasmid pIPX61 that has been deposited at the CNCM on May 14, 1996 under the Accession Number I-1705.

8. The recombinant vector according to Claim 6, which is plasmid pIPX30 that has been deposited at the CNCM on February 13, 1`997 under the Accession Number I-1845.

9. A recombinant cell host containing a purified polynucleotide according to any one of Claims 1 to 5 or a recombinant vector according to any one of Claims 6 to 8.

10. The recombinant cell host according to Claim 9 which is a mycobacterium cell host belonging to the Mycobacterium tuberculosis complex.

11. The recombinant cell host according to Claim 10 which is Mycobacterium tuberculosis.

12. The recombinant cell host according to Claim 10 which is Mycobacterium bovis-BCG.

13. The recombinant cell host according to Claim 9 which is the E. coli strain deposited at the CNCM on May 14, 1996 under the Accession Number I-1705.

14. The recombinant cell host according to Claim 9 which is the E. coli strain deposited at the CNCM on February 13, 1997 under the Accession Number I-1845.

15. A recombinant cell host containing a polynucleotide of SEQ ID NO 2 or a recombinant vector carrying SEQ ID NO 2 which is Mycobacterium smegmatis.

16. A purified polypeptide expressed by a recombinant cell host according to ~~any one of Claims 9 to 13 and 15~~ Claim 9.

17. A purified polypeptide of Claim 16 which was chosen from the group of polypeptides consisting in:

a) a polypeptide which comprises the following amino acid sequence of SEQ ID NO 5:

MAEMKTDAATLGQEAGNFERISGDLKTQIDQVESTAGSLQGQWRGAAGTAA QAAVVRFQEAANKQKQELDEISTNIRQAGVQYSRADEEQQQALSSQMGF;

b) a polypeptide comprising:

i) amino acid in position 1 to amino acid in position 48 of SEQ ID NO 5; or

ii) amino acid in position 60 to amino acid in position 100 of SEQ ID NO 5;

c) a poly peptide comprising at least one antigenic portion of a polypeptide a) or b).

18. An oligomeric polypeptide comprising at least two units of a polypeptide according to Claim 17.

19. The oligomeric polypeptide of Claim 18 comprising up to 10 units of a polypeptide according to Claim 17.

20. A purified polypeptide comprising at least one antigenic portion of a polypeptide according to Claim 17.

21. The purified polypeptide according to Claim 18 wherein the antigenic portion of the polypeptide of sequence SEQ ID NO 4 is chosen among the group consisting in the following antigenic portions:

a) SEQ ID NO 6:

NH2-MAEMKTDAATLGQEAGNFERISGDLKTQIDQVESTAGS LQGQWRGAAGT-COOH;

b) SEQ ID NO 7: NH2-QEAANKQKQELDEISTNIRQAGVQYSRADEEQQQ ALSSQMGF-COOH;

c) SEQ ID NO 8: NH2-QEAGNFERISGDLKTQIDQV-COOH;

d) SEQ ID NO 9: NH2-GDLKTQIDQVESTAGS-COOH;

e) SEQ ID NO 10: NH2-GSLQGQWRGAAGTAAA-COOH;

f) SEQ ID NO 11: NH2-QEAANKQKQELDEIST-COOH;

g) SEQ ID NO 12: NH2-STNIRQAGVQYSRADEEQQQALSSQMGF-COOH;

h) SEQ ID NO 13: NH2-RADEEQQQALSSQMGF-COOH.

22. The purified polypeptide according to ~~any one of Claims 20 and 21~~ Claim 20 comprising from 2 to 10 antigenic portions of the polypeptide of SEQ ID NO 4.

23. A purified polypeptide or an oligomeric polypeptide according to ~~any one of Claims 16 to 23~~ Claim 16 which is under the form of a MAP construct.

24. A purified polypeptide or an oligomeric polypeptide according to ~~any one of Claims 16 to 23~~ Claim 16 which comprises an additional T-epitope.

25. A monoclonal or a polyclonal antibody directed specifically against a purified polypeptide or an oligomeric polypeptide according to ~~any one of Claims 16 to 24~~ Claim 16.

26. An immunogenic composition comprising a purified polypeptide or an oligomeric polypeptide according to ~~any one of Claims 16 to 24~~ Claim 16.

27. A vaccine composition comprising a purified polypeptide or an oligomeric polypeptide according to ~~any one of Claims 16 to 24~~ Claim 16.

28. The vaccine composition according to Claim 27 wherein said vaccine composition comprises additionally an antigenic protein from Mycobacterium tuberculosis or an antigenic portion of an antigenic protein from Mycobacterium tuberculosis.

29. The vaccine composition according to Claim 28 wherein said vaccine composition comprises additionally the ESAT-6 antigenic protein or an antigenic portion of the ESAT-6 protein.

30. A diagnostic method for detecting the presence of a Mycobacterium tuberculosis bacterium in a biological sample, said diagnostic method comprising the steps of:

a) bringing into contact the biological sample expected to contain a given pathogenic microorganism with a purified monoclonal or polyclonal antibody according to Claim 25;

b) detecting the antigen-antibody complexes formed.

31. A diagnostic method for detecting the presence of a Mycobacterium tuberculosis bacterium in the serum of an infected patient, said diagnostic method comprising the steps of:

a) bringing into contact the serum sample expected to contain a given pathogenic microorganism with a purified polypeptide or an oligomeric polypeptide according to ~~any one of Claims 16 to 24~~ Claim 16;

b) detecting the antigen-antibody complexes formed.

32. A diagnostic kit for the in vitro diagnosis of an infection by Mycobacterium tuberculosis, comprising the following elements:

a) a purified preparation of a monoclonal or a polyclonal antibody according to Claim 25;

b) suitable reagents allowing the detection of the antigen/antibody complexes formed, these reagents preferably carrying a label compound, or being recognized themselves by a labeled reagent;

c) optionally a reference biological sample containing the Mycobacterium tuberculosis antigen recognized by the purified monoclonal or polyclonal antibody (positive control);

d) optionally, a reference biological sample that does not contain the Mycobacterium tuberculosis antigen recognized by the purified monoclonal or polyclonal antibody (negative control).

33. A diagnostic kit for the in vitro diagnosis of an infection by Mycobacterium tuberculosis, comprising the following elements:

a) a purified preparation of a purified polypeptide or an oligomeric polypeptide according to ~~any one of Claims 16 to 24~~ Claim 16;

b) suitable reagents allowing the detection of the antigen/antibody complexes formed, these reagents preferably carrying a label compound, or being recognized themselves by a labeled reagent;

c) optionally, a reference biological sample containing a polyclonal or monoclonal antibody recognizing the purified polypeptide or the oligomeric polypeptide of step a) (positive control);

d) optionally, a reference biological sample that does not contain a polyclonal or monoclonal antibody recognizing the purified polypeptide or the oligomeric polypeptide of step a) (negative control).

34. A method for detecting Mycobacterium tuberculosis is a biological sample comprising the steps of:

a) bringing into contact a purified polynucleotide according to ~~any one of Claims 1 to 3~~ claim 1 with a biological sample;

b) detecting the hybrid nucleic acid molecule formed between said purified polynucleotide and the nucleic acid molecules contained within the biological sample.

35. The method of Claim 34, wherein before step a), the nucleic acid molecules of the biological sample have been made available to a hybridization reaction.

36. A method for detecting a bacterium belonging to the Mycobacterium tuberculosis complex or to Mycobacterium bovis in a biological sample comprising the steps of:

a) bringing into contact a purified polynucleotide according to ~~any one of Claims 1 to~~ claim 1 3 that has been immobilized onto a substrate with a biological sample.

b) bringing into contact the hybrid nucleic acid molecule formed between said purified polynucleotide and the nucleic acid contained in the biological sample with a labeled polynucleotide according to ~~any one of Claims 1 to 3~~ claim 1, provided that said polynucleotide and polynucleotide of step a) have non-overlapping sequences.

37. The method of claim 36, wherein, before step a), the nucleic acid molecules of the biological sample have been made available to a hybridization reaction.

38. The method of any one of Claims 36 or 37, wherein, before step b), the nucleic acid molecules that are not hybridized with the immobilized purified polynucleotide are removed.

39. A method for detecting a bacterium belonging to the Mycobacterium tuberculosis complex in a biological sample comprising the steps of:

a) bringing into contact the nucleic acid molecules contained in the biological sample with a pair of purified polynucleotides according to ~~any one of Claims 1 to 3~~ claim 1;

b) amplifying said nucleic acid molecules;

c) detecting the nucleic acid fragments that have been amplified, for example by gel electrophoresis or with a labeled polynucleotide according to ~~any one of Claims 1~~ Claim 1 to 3.

40. The method of Claim 39, wherein before step a), the nucleic acid molecules of the biological sample have been made available to a hybridization reaction.

41. A kit for detecting a bacterium belonging to the Mycobacterium tuberculosis complex or to Mycobacterium bovis in a biological sample comprising:

a) a purified polynucleotide according to ~~any one of Claims 1 to 3~~ Claim 1;

b) reagents necessary to perform a nucleic acid hybridization reaction.

42. A kit for detecting a bacterium belonging to the Mycobacterium tuberculosis complex or to Mycobacterium bovis in a biological sample comprising:

a) a purified polynucleotide according to ~~any one of Claims 1 to 3~~ Claim 1 that is immobilized onto a substrate;

b) reagents necessary to perform a nucleic acid hybridization reaction;

c) a purified polynucleotide according to ~~any one of Claims 1 to 3~~ Claim 1 which is radioactively or non-radioactively labeled, provided that said polynucleotide and the polynucleotide of ~~step a)~~ have non-overlapping sequences.

43. A kit for detecting a bacterium belonging to the Mycobacterium tuberculosis complex or to Mycobacterium bovis in a biological sample comprising:

a) a pair of purified oligonucleotides according to ~~any one of Claims 1 to~~ Claim 1 3;

b) reagents necessary to perform a nucleic acid amplification reaction;

c) optionally, a purified polynucleotide ~~according to any one of claims~~ s useful as a probe.

44. A recombinant vector according to Claim 6, which is plasmid pIPX26 that has been deposited at the CNCM on May 14, 1996 under the Accession Number I-1706.

45. A recombinant vector according to Claim 6, which is plasmid pPX1 that has been deposited at the CNCM on May 14, 1996 under the Accession Number I-1707.

46. A recombinant cell host according to Claim 9, which is the E. coli strain that has been deposited at the CNCM on May 14, 1996 under the Accession Number I-1706.

47. A recombinant cell host according to Claim 9, which is the E. coli strain that has been deposited at the CNCM on May 14, 1996 under the Accession Number I-1707.

48. The vaccine composition according to Claim 27 comprising a recombinant cell host containing a polynucleotide encoding a polypeptide according to Claim 1 or a recombinant vector containing said polynucleotide.

49. The vaccine composition according to Claim 48, wherein said polynucleotide or said vector encodes both the lhp or the ESAT-6 antigenic polypeptides or antigenic portion thereof.

50. The vaccine composition according to Claim 48 comprising a recombinant cell host expressing lhp and a recombinant cell host expressing ESAT-6.

51. The vaccine composition according to ~~any one of Claims 48 to 50~~ Claim 48, wherein the recombinant cell host is an eukaryotic cell host.

52. The vaccine composition according to ~~any one of Claims 48 to 50~~ Claim 48, wherein the recombinant cell host is a prokaryotic cell host.

53. The vaccine ~~composition~~ according to Claim 52, wherein the recombinant cell host is chosen from the group of bacteria consisting in:

a) an attenuated bacterium belonging to the tuberculosis-complex;

b) E. coli;

c) a bacterium belonging to the Salmonella genus;

d) a bacterium belonging to the Pseudomonas genus.

54. A polynucleotide useful as a primer or a probe according to Claim 1 which is chosen from the group consisting in:

a) SEQ ID NO 14: 5'-CTGCAGCAGGTGACGTCGTTG-3'

b) SEQ ID NO 15: 5'-CCGGGTGGCCGGGAAGTCTGTGT-3'

c) SEQ ID NO 16: 5'-ACTACTTTCTCTTTCTACCTTCC-3'

55. A pair of oligonucleotide primers according to Claim 54, which is chosen from the group consisting in:

a) SEQ ID NO 14 and SEQ ID NO 15;

b) SEQ ID NO 14 and SEQ ID NO 16.